SEROLOGICAL PREGNANCY TEST USING LATEX PARTICLES

AN ASSESSMENT OF ITS RELIABILITY AND CONVENIENCE

BY

N. R. GARDEN,* M.A., B.M., B.Ch., D.Path. H. MILLER, M.D., M.R.C.P.

J. A. DURANT, B.Sc.

Portsmouth and Isle of Wight Area Pathological Service, Royal Portsmouth Hospital

Hitherto the laboratory diagnosis of early pregnancy has depended on the use of "biological" techniques. The presence in the urine (or serum) of human chorionic gonadotrophin (H.C.G.) is demonstrated by its physiological effect in a variety of animals.

The first of these techniques to be introduced, the Aschheim–Zondek test (Aschheim and Zondek, 1928), involved the injection of a sample of the patient's urine into immature female mice. This test was followed by the Friedman (1929) test, in which mature non-pregnant female rabbits were used. In both these animals the ovaries were examined after a suitable interval and the presence of haemorrhagic follicles indicated a positive result.

At the present time frogs or toads, both male and female, are probably in more common use than mice or rabbits for the detection of H.C.G. They can be used repeatedly and are less troublesome to keep and handle. Even so, some method quicker and more compact than the biological type of test was clearly desirable, and, since it had been known for a long time that H.C.G. had antigenic properties, several workers using highly purified preparations of H.C.G. developed potent and specific antisera in rabbits. Brody and Carlström (1960, 1961) used their antisera to determine H.C.G. concentration chiefly in serum by a complement-fixation test. McKean (1960) employed a precipitin test, while Wide and Gemzell (1960) described an erythrocyte-flocculation inhibition reaction, both tests being intended for use with urine. A clinical test based on the latter reaction has been described by Fulthorpe, Parke, Tovey, and Monckton (1963).

Recently a neutralization test using polystyrene latex particles coated with H.C.G. has been made available in kit form.† It is rather more simple than the other immunological techniques and has obvious attractions for laboratories with insufficient space and heavy routine commitments. Our laboratory is responsible for pregnancy-testing for a large area of South Hampshire, the Isle of Wight, and part of West Sussex, and approximately 2,000 tests are carried out each year. The female frog *Xenopus laevis* is used as the test animal, and, to reduce expense, breeding is carried out in the laboratory, in preference to buying frogs elsewhere. The space and technical working time required are considerable and it was clearly desirable to replace the xenopus (Hogben) test with the latex test (as it will now be called) if this could be done without a significant lessening in reliability.

Accordingly a comparative series of tests was immediately undertaken to verify the claims made for the new method.

Material and Methods

Latex Test.—The test pack contains 10 disposable test-tubes, a turbidity standard, and two reagents—one consists of the antigen (a suspension of latex particles sensitized with H.C.G.), the other of a rabbit H.C.G. antiserum. The antiserum is standardized so that, under test conditions, 0.5 ml. will agglutinate 1 ml. of the antigen suspension. The manufacturers suggest the following requirements in the urine samples: (1) they should be early-morning specimens, taken between 6 and 12 weeks after the last menstrual period (L.M.P.); (2) they should have a specific gravity of 1015 or over and be free of more than a trace of salicylates (which may lead to a false-positive reaction); and (3) they must be tested (or frozen at −20° C. and stored) within 12 hours.

The technique is as follows. The urine is centrifuged at high speed (3,000 r.p.m. or more) for three minutes. 0.5 ml. of rabbit H.C.G. antiserum is pipetted into a disposable test-tube and 0.5 ml. of supernatant urine is added, with thorough mixing. The tubes are then placed in a 37° C. water-bath for one hour. Then 1 ml. of the H.C.G.-coated latex suspension, thoroughly shaken, is added to each tube, with thorough mixing. The tubes are incubated in the water-bath for a further two hours. At the end of this total incubation time of three hours the tubes are centrifuged for two minutes at 1,000 *g* (approximately 3,000 r.p.m. in most small centrifuges). The time and speed are critical and a reliable tachometer should be used on all occasions. A saline control tube is put up with each batch of tests.

If the urine contains sufficient H.C.G. the rabbit antiserum is neutralized and is unable to agglutinate the sensitized latex particles. These accordingly cannot be deposited by the standard centrifugal force exerted, so that the tube remains turbid and a positive result is recorded. Conversely, in negative tests the unneutralized antiserum agglutinates the latex particles and a clear or almost clear supernatant is obtained by centrifuging. These two reactions are differentiated by the slightly turbid standard suspension, the test and standard tubes being viewed conveniently in front of a wide horizontal black line on the white box of the test kit.

Xenopus Test.—The method of extracting H.C.G. from urine is essentially that described by Hobson (1952), and needs only a very brief outline here. 60 ml. of urine are used in an extraction process employing kaolin adsorption and the final extract of H.C.G. has a volume of 5 ml. Half this amount is injected into a female South African clawed frog (*Xenopus laevis*), and if ovulation takes place about 18 to 24 hours later a positive result is reported. If no ovulation occurs, the second half of the extract is injected into a frog which is known to have given a positive reaction in its previous test. If this frog does not ovulate then a negative report is issued.

Organization of Main Trial.—A circular letter was sent to the senior obstetricians and to the general practitioners (about 100) in the area from which the requests were normally received. This asked for their co-operation in the trial by arranging delivery of the urine within 12 hours of passing if possible, by giving brief clinical details—for example, date of L.M.P. and drugs taken in the previous

*Now in the Department of Morbid Anatomy, St. George's Hospital, London.

†Ortho pregnancy test kit, Ortho Pharmaceutical Corporation, High Wycombe, Bucks.

48 hours—and by completing in each case the cards which are sent out routinely two months after the test. These cards enable the practitioner to indicate by a circle round the answer whether his patient is "pregnant," "not pregnant," or has "miscarried" after the test.

Results

Main Comparative Series

Specimens of urine accepted for this series had to conform to the conditions already described—that is, they were less than 12 hours old, had an S.G. of 1015 or over, and were negative when tested for salicylates by the ferric chloride method. However, no specimen was rejected because it fell outside the range of 6 to 12 weeks from the L.M.P. or because it had only a very imprecise timing from the L.M.P.

Every eligible specimen was examined by the latex test and the Hogben test. The staff concerned worked independently of one another on each specimen until the written report from each method was available. A total of 233 urine specimens were tested in this manner.

It proved difficult to recover a really high proportion of final clinical reports. Rather more than half of the total number of cards arrived in the normal way. Some others were readily obtained after inquiry (in nearly all these cases the practitioners had lost contact with the patient and were hoping to be able to obtain a reliable answer later). There remained a considerable number of patients who had moved from the district without leaving a forwarding address. Eventually, 159 cases in which the clinical result was known were available for analysis.

The Table shows the accuracy of diagnosis achieved by the two methods and analyses the cases incorrectly diagnosed.

Accuracy of Diagnosis

Type of Test	No. of Cases	Correct Reports		No. of Incorrect Results	False-negative Results	False-positive Results
		No.	%			
Xenopus	159	148	93·1	11	11	None
Latex ..	159	144	90·6	15	10	5

In addition to the main trial, the absolute sensitivity of the test was estimated and some aspects of the standard technique were examined.

Absolute and Comparative Sensitivity of the Latex Test

The latex technique was tested for sensitivity by the use of a purified extract of H.C.G. ("pregnyl"), standardized in international units. A wide range of dilutions was prepared in saline, including concentrations of 50, 30, 20, 10, 5, and 2 I.U./ml. By the standard technique the tubes containing 20 I.U./ml. and more were positive while the weaker concentrations were negative. This titration was repeated on several occasions, and always when a new batch of reagents was brought into use. It was not found to alter, and it is reasonable to assume that the latex test can detect H.C.G. in a minimum concentration of approximately 20 I.U./ml.

By means of a urine sample which had reacted positively in both techniques the sensitivities of the two methods were compared. The following dilutions of the urine were made in saline and used in the standard latex test: 1/1, 1/2, 1/4, 1/6, 1/8, and 1/10. The 1/2 dilution was the lowest concentration to react positively and would therefore contain approximately 20 I.U. of H.C.G./ml. The other part of the specimen was used to prepare an extract for the xenopus test in the normal way and the following dilutions were made from it: 1/10, 1/25, 1/50, and 1/75.

One pair of frogs was used to test each of the four dilutions. Both frogs which had received the 1/25 dilution reacted positively while those receiving the 1/50 dilution showed no reaction. The positive thresholds (1/2 dilution for the latex test and 1/25 for the xenopus test) show that the xenopus technique appears to be *10 to 15 times more sensitive* than the latex test and, in absolute terms, should detect a concentration of approximately 2 I.U. of H.C.G./ml.

It is, of course, unsatisfactory to use such a small number of animals for this test, but we were unable to spare more owing to the routine requirements of the laboratory. Nevertheless Hobson (1952), in a very detailed examination of all aspects of the xenopus test, suggests that the frogs are sensitive to a concentration of 3 I.U. of H.C.G./ml. and so it appears that the xenopus technique, as applied in our laboratory, achieves a similar degree of sensitivity.

Modifications of Technique

A small series was tested in which the urines, while conforming to the strict criteria in other respects, gave a positive reaction for salicylates (ferric chloride test). The series reached a total of 22, by which time it was evident that the supernatant of some of the supposedly negative tubes was less clear than in the normal run of negative tubes in the main series. To verify this point, three male subjects took 10 gr. (650 mg.) of aspirin ("paynocil") in the evening, followed by a similar dose the next morning. They passed a test specimen two hours later. All three specimens reacted strongly with ferric chloride reagent and all of them, when tested, produced a supernatant the turbidity of which was not much less than that of the standard. In spite of this it was possible to classify them with confidence.

Another small series was designed to see whether specimens could be tested with confidence if they were more than 12 hours old. Fifteen routine samples were investigated by the latex method within the 12-hour limit. They were then kept on the bench at room temperature and retested after five days (any extra turbidity due to bacteria being removed by centrifuging in the normal way). Of these 15 specimens, seven were originally positive and remained so when retested, and seven remained negative in the second test. The remaining one was recorded as "just negative" originally; on the fifth day it was so similar to the standard in turbidity as to be considered "positive." On a later occasion eight further cases were similarly tested, within 12 hours and on the seventh day. Two of these were consistently negative and five consistently positive. The eighth sample, originally classified as "just positive," was read as "just negative" on the seventh day.

It seemed desirable, for the sake of economy, to see whether the test could be scaled down without incurring any disadvantages. Volumes of the reagents and urine were reduced to one-fifth of those recommended and the reagents were placed in disposable plastic tubes 5 by 0.5 cm. The standard suspension was sealed in a similar tube. Seventeen specimens were handled in this way until it became clear that if these narrow-bore tubes did not lie quite accurately along the radial axis during centrifuging, one wall of the negative tubes became "tattooed" with some of the latex aggregates. Thus the apparent turbidity varied with the rotation of the tube. Moreover the turbidity standard was difficult to resuspend in the narrow plastic tube, even when an ample air-space was left. These

were serious drawbacks which caused us to abandon the experiment.

Discussion

Before the major features of the latex test are discussed it will be convenient to review the question of technical modifications.

Our tests have shown that even modest therapeutic doses of aspirin cause an increase in the turbidity of the negative tubes. Inevitably there will be a proportion of cases in which the test will be read as negative only with difficulty, and perhaps on occasion a false-positive reading will result. We feel that the manufacturers' warning on this point should be strictly observed and we would not, in this laboratory, accept for the latex test any specimen which showed a positive reaction for salicylates.

Experience with specimens kept at room temperature for some days suggests that no change of reading is likely over a period of at least 48 to 72 hours. When we studied this aspect of the technique we had in mind the problems of our own area. The xenopus test has features which make centralization necessary, so that, as we stated earlier, this laboratory carries out pregnancy tests for a large district. Many of our early-morning specimens of urine arrive by post and are inevitably more than 24 hours old. These could probably be tested safely by the latex method. However, since the latex test needs little space and requires apparatus which is available in any non-specialized laboratory, the logical solution, if this test is adopted, is to decentralize. There would then be no occasion to test urines more than 12 hours old.

We are doubtful about the value of developing micro-methods for this test and do not intend to persevere. Doubtless workers in other laboratories have developed or will develop reduced-volume versions of the present technique which are as reliable though perhaps not as convenient. But it can be argued that no reduction in accuracy and convenience should be tolerated in the development of small-scale methods unless their introduction facilitates the testing of biological material available only in small volumes.

Latex Test as a Replacement for the Xenopus Test

If the latex test is to supersede the biological test, it must be assessed partly on general convenience (including space requirements, speed, and cost), but most of all on reliability. In convenience the latex test is greatly superior. It requires simple apparatus and little working space. If necessary, results can be produced in four hours, and for a batch of 10 tests the working time for one man will be about 45 minutes. In contrast, the xenopus test needs 24 to 48 hours for an answer and the approximate technical time required is two hours for a batch of 10 tests.

The standard of accuracy achieved with the frog tests differs somewhat in the various series reported. The Ortho Research Foundation (1961) record an accuracy of 97.99% in 1,193 biological tests using frogs. Hobson (1952), using the xenopus test, gives a figure of 99.8% correct results in 37,020 cases. In our present series correct results with the xenopus test amounted to 93.1% of the total. Some of the discrepancy is due presumably to variations in technique (such as general animal handling and the exact interval between injections). But a more important factor, probably, is the readiness (or the reverse) with which a laboratory accepts vague or non-existent details of the L.M.P. A non-selective policy will, of course, increase the proportion of false-negative reports from cases falling outside the ideal range of 6 to 12 weeks after the L.M.P.

For this reason the worth of a possible replacement test can only be fairly assessed in direct comparison with the accuracy of the biological test as it is performed in the same laboratory. In the present comparative series of 159 cases the latex test gave an accuracy of 90.6% (compared with 93.1% for the xenopus test). The Ortho Research Foundation (1961), in a series of 1,390 cases tested by their latex method, report an accuracy of 97.77% (compared with 97.99% for their series of frog tests). Goldin (1962), describing his experience with the latex test, reports, as one part of his study, 69 cases in which an adequate clinical follow-up was possible; 39 specimens of urine reacted positively and 30 negatively. The total numbers of correct answers were 37 and 29 respectively. This makes the overall accuracy of his test 95.7%. The absolute accuracy of his frog testing (which was undertaken in a larger comparative series) is not given, as he was not concerned with this aspect.

It is, of course, important, in comparing the accuracy of a test in several independent series, to have the ratio of true positives to true negatives approximately the same, in case the test has a bias toward false-negative or false-positive results. Our series contains 60% true positive cases and Goldin's 55%.

If the overall accuracy alone of the latex test is considered, the technique would appear to be equally satisfactory for routine pregnancy testing. The fact that a method 10 to 15 times less sensitive than the xenopus test performs almost equally well in practice is readily explained by the pattern of excretion of H.C.G. in early pregnancy. Owing to the obvious inconvenience of obtaining frequent 24-hour specimens of urine from normal women in early pregnancy, information is based on small numbers of subjects. Smith, Albert, and Randall (1951) investigated in detail 17 women, six of whom had entirely normal pregnancies. They found that between day 24 and day 40 (after the L.M.P.) there was a "pre-elevation" plateau of 1,000–5,000 I.U. of H.C.G./24 hours. Between day 40 and day 90 there was a rapid and considerable (but variable) rise to a peak, followed after this period by a fall to a "post-elevation" plateau ranging from 2,000 to 15,000 I.U./24 hours. It seems that the rate of H.C.G. excretion rises so rapidly that it comes within the sensitivity range of both frog and latex methods by the sixth week after the L.M.P.

Examination of the incorrect answers in our comparative series raises a crucial issue. It is well known to pathologists and clinicians who make use of it that the xenopus test very rarely produces a false-positive answer. Hobson (1952), in his considerable experience with the method, did not personally come across a single false-positive result. There have been only two such cases in our laboratory in 12 years (some 30,000 tests). In the present series the xenopus test produced 11 false-negative results, whereas the latex test gave 10 false-negative and five false-positive answers.

This means that for those who make clinical use of the reports the new test involves a qualitative change influencing interpretation. It is very readily assumed in laboratories that it is a desirable feature in a pregnancy test never to give a false-positive reaction. In our view this assumption needs careful re-examination. Indeed, it may merely represent the process of making a virtue out of an inherent property of the test. In other investigations it is important that a screening test should be sensitive enough to select all possible cases for close follow-up, a process which clearly implies a proportion of false positives.

It must be pointed out that no extra-sensitive routine test (giving a risk of false positives only) is available as

yet. The occurrence of false positives in the latex test does not, we know, imply a greater degree of sensitivity—rather, it seems to be due to some random factor not yet identified. Goldin (1962) encountered two false positives in his 69 cases. The Ortho Research Foundation (1961) differ from us in their much bigger series. Their latex testing gave 2.09% false-negative and 0.14% false-positive reactions. Conversely, their frog tests gave values of 0.50% and 1.51% respectively.

It is clearly necessary for the clinicians who request tests for pregnancy to agree with the pathologists performing these tests about the limits of accuracy that should be acceptable.

So far as the laboratory is concerned there is much to recommend a tube test which is of approximately equal effectiveness to the more laborious biological tests. However, our limited experience shows that if the latex test is adopted, an appreciable number of false-positive results must be expected, a situation which hitherto has not had to be considered.

We are reluctantly unable to recommend the latex test to our clinical colleagues unless they are prepared to accept the possible consequences of the false-positive results.

There is one aspect of the latex test which may, however, be used to advantage, even though it is not used for pregnancy diagnosis. To confirm the diagnosis of chorion-epithelioma and other moles it is usual to carry out a quantitative biological test with urine dilutions. The quantitative Hogben test (Hobson, 1952) uses six toads and is therefore wasteful of animals and time. We believe that the latex test may be valuable here, but have not had the opportunity to try it.

Summary

Two methods of testing urine for pregnancy are compared. One is a recently introduced sensitized latex particle precipitation test, and this is compared with the widely used Hogben test—a biological test using *Xenopus laevis* female frogs.

Early-morning urine samples from 233 women were examined by the two methods. In 159 of these cases it was possible to determine whether or not the results obtained were correct.

The latex test was accurate in 144 (90.6%) cases and the Hogben test in 148 (93.1%) cases.

The Hogben test did not make any false-positive predictions, whereas the latex test did so in a third of the incorrectly predicted cases (5 out of 15).

The latex test was found to be sensitive to a minimum concentration of 20 units of H.C.G./ml. and the Hogben test to a concentration of approximately 2 units of H.C.G./ml.

It is suggested that the latex test may be of value for the quantitative determination of H.C.G. in urine.

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ANKYLOSING SPONDYLITIS AND CHRONIC INFLAMMATORY DISEASES OF THE INTESTINES

BY

J. A. McBRIDE,* M.B., M.R.C.P.Ed. **MARGARET J. KING, M.B., M.R.C.P.Ed.**

A. G. BAIKIE†, M.B., F.R.C.P.Ed.

Medical Research Council Clinical Effects of Radiation Research Unit, Edinburgh

G. P. CREAN, M.B., M.R.C.P.Ed. **W. SIRCUS, M.D., Ph.D., F.R.C.P.Ed., M.R.C.P.**

Gastro-intestinal Unit, Western General Hospital, Edinburgh

A number of reports have been published in the past few years which note an association between ankylosing spondylitis and inflammatory diseases of the bowel. Many of these reports are of patients who, in addition to ankylosing spondylitis, had either Crohn's disease or ulcerative colitis. In one series of 177 male patients Romanus (1953) found that three had ulcerative colitis, and Steinberg and Storey (1957) reported six cases, all with ankylosing spondylitis, of whom four had ulcerative colitis, one had Crohn's disease, and one had both Crohn's disease and ulcerative colitis. Among 220 cases of ankylosing spondylitis, Wilkinson and Bywaters (1958) described four with ulcerative colitis and one with Crohn's disease; and Fernandez-Herlihy (1959), in a review of 555 cases of ulcerative colitis, found 28 patients with ankylosing spondylitis. In a study of 13,352 patients who had received radiotherapy for ankylosing spondylitis an apparent excess of deaths ascribed to ulcerative colitis was found (Court Brown and Doll, 1957, and personal communication). Thus up to December, 1955, 13 deaths from ulcerative colitis had occurred where 0.65 might have been expected.

This paper reports further instances of the association of inflammatory diseases of the gastro-intestinal tract with ankylosing spondylitis. In addition, attention is drawn to a familial predisposition to the development of these diseases, alone or in combination.

Clinical Material

Two groups of hospital patients were investigated. The larger, group A, consisted of 870 patients who had been treated with radiotherapy for ankylosing spondylitis in Edinburgh between 1935 and 1960. The smaller group (B)

*Present address: Department of Medicine, Hammersmith Hospital, London.
†Present address: University of Melbourne, Department of Medicine, St. Vincent's Hospital, Victoria, Australia.